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# Bioethanol Production by Cofermentation of Hexose and Pentose from Algal Feedstock using Pichia Stiptis and Candida Shehatae

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**Abstract**

Due to continuous increase in fuel prices, scientists and researchers have shifted their attention towards the use of biofuels as the renewable source of energy. Now days, bioethanol production is not a big approach but when using some renewable source as a feedstock for biofuel production. They may affect in fuel world so bioethanol from algal feedstock are the "3rd generation" of biofuel to be known as one of the most important renewable energy sources. Today, global demand of bioethanol arise the opportunity to explore new renewable biomass sources. Microalgae are a high source of carbohydrate of various forms so they provide viable feedstock for ethanol fermentation. Algal feedstock's have shorter growth cycle as compare to other plants, hence the algae is a very advance source of biomass for the production of biofuels and also reduce in climate changing effects. The purposes of this research are to produce ethanol from algal biowaste using pre-treated with alkali pretreatment ($H_2SO_4$), enzymatic hydrolysis and anaerobic fermentation. After optimization of physical and cultural parameters isolated microbial culture *pichia stiptis* and *candida shehatae* were used for co fermentation process. This research investigated co-fermentation technique by using *pichia stiptis* and *candida shehatae* strain in 5L batch bioreactor to produce bioethanol. A optimized medium containing 60 g/L glucose and 30 g/L xylose at 30°C and PH 6.5 was agitated 120 rpm to 280 rpm. Cofermentation was achieved by *pichia stiptis* and *candida* in the medium containing the glucose and xylose mixture. *Pichia Stiptis* fermented both sugars, produce 20 g/L ethanol and consumed 80 % of xylose in 12h to 180h at a utilization rate of 0.20 g/L in the presence of glucose. After optimization of all physical parameters isolated culture directly transferred in pretreated algal feedstock supplemented with medium compositions. Co- fermentation can be economically applied to utilize glucose and xylose and produce a high ethanol yield and this proposed technique can be used for industrial ethanol production.

**Keyword:** Algal biomass, Bioethanol, Fermentation, Optimization, Pretreatment.

## I. Introduction

Biomass is the most promising renewable source of energy because it can be used as an alternative source for bio-energy production. Natural resources for energy production are becoming extinct day by day. The main reasons behind this biomass energy production is that it can be produced from wood, plant and animal wastes, forestry wastes which indicate that biomass can be produced from those materials that are regarded as wasted materials which are again re-used and convert into bio-energy. Biomass does not emit any harmful gases and produced abundant and renewable clean energy, and reduces the usage of fossil fuels for bio-energy production and also it can be used to create different products. The main reason behind the biomass usage is it reduces emission of greenhouse gases. Usage of biomass utilization for biofuel production will grow within the coming years. The clean electricity generation will be enough for more than 17,000 UK householders a year and the usage of renewable for electricity generation in UK is increased by 60 percent and the share of electricity is around 9.7 percent in 2012 and 15.5 percent in 2013. Now a day's around 3.9 million tonnes



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of biomass, mostly in the form of woodchips, straw form and pellets, were burnt to generate electricity during those 12 months. Usage of biomass for biofuel production will grow exponential benefits within coming years. The market values of electricity generated from biomass in the United States was over $45 billion in 2011. About 70 percent of all biomass in the world is used in the residential sector, 14 percent is used in industry and 11 percent is transformed into electricity, heat, or energy such as liquid fuel or biogas. In 20$^{th}$ century algae to biofuel opportunity is Abundant, affordable, and sustainable feedstocks are the lifeblood of the burgeoning biofuels industry today. Algae is a broad and diverse group of simple autotrophic organisms ranging from unicellular to multicellular forms, constitute the largest part of the biotic components of aquatic ecosystems. However, selection of appropriate algal species, standardization of mass cultivation practices coupled with development of economically viable compounds and optimization of hydrolysis and fermentation process are likely to play a major role in the improved production of ethanol. Algae must be considered as part and parcel of the feedstock mix for producing advanced biofuels. In contrast to the development of cellulosic biofuels which has benefit from a direct agricultural and process engineering lineage, there is no parallel agricultural enterprise equivalent for cultivating algae at a similar scale. A high strategically structured investment to tackle the challenges of algal biofuels is thus needed to support commercialization activities.

## II. Materials and Methods

### Isolation and screening of xylose - glucose utilizing and ethanol producing microorganisms:

#### Collection and enrichment of samples

For isolation of xylose and glucose utilizing microorganisms various sample were collected. Samples were collected from soil sample, blackberries, banana, cattle dung, goat and pig excreta using xylose-glucose enriched medium. Appropriate dilution of the samples in water were placed on pre-anaerobic YPEXG medium containing g/L xylose and glucose ; xylose 30 ; glucose 60; yeast extract 10; peptone 20 ; supplemented with ampicillin (50mg/ml) and maintained pH 6.5 ± 0.2. Morphologically different colonies were picked up and isolated a pure colonies by streaking them the plates containing the same medium.

**2.4.1**

**Table 1. YEPXG medium compositions**.

| YEPXG Medium | Composition gL$^{-1}$ |
|---|---|
| Yeast Extract | 10 |
| Peptone | 20 |
| Xylose<br>Glucose | 30<br>60 |

#### Selected colonies transfer into fresh YPXG medium

The pre-anaerobic medium was sterilized by autoclaving at 120 c for 15 min. D – xylose and D – glucose were autoclaved separately at 110°C for 10 min. After sterilization and cooling, the solutions were mixed to form a complete medium prior to inoculation. A loopful of 24 h old yeast isolate was transferred to 50 ml of the sterilized pre-anaerobic medium in serum bottle and incubated at 30°C on a rotary shaker at 120 rpm. The cells were grown for 24 hrs.

#### Inoculum preparation

The 5 ml of this culture was transferred to 100 ml pre-anaerobic YEPXG medium of the same composition in 250 ml serum bottle. The culture was grown again for 24 hrs under conditions similar to those describe above and the broth was centrifuged at 10,000 rpm for 10 min. The cell pellet was washed and suspended in 100 ml sterile distilled water and check cell density in spectrophotometer.

#### Preparation of substrates

Fresh water-hycianth and azolla plants with long stem were collected natural resources and washed to remove adhering dirt and chopped in small pieces, dried in sunlight and make powdered.

#### Acidic Pretreatment

Dilute acid pretreatment was used for hydrolysis process, 10 g dried azolla and water hycianth was mixed with 20 ml of 2 %(v/v) sulfuric acid, and autoclaved 121°C for 60 min., followed by neutralization with concentrated NaOH (4-8 M) to pH 4 to 5.



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After pretreatment concentration of total sugars was analyzed by spectrophotometer using DNS method and Agilent HPLC equipment.

### III. Batch fermentation

Prepared inoculums of *pichia stiptis* and *candida* used for inoculation of 3L concentrated hemicelluloses acid hydrolyses supplemented with the defined medium gradients. Bioreactor was inoculated with 8 % (v/v) of isolated strains. Fermentation were carried out in a shake flask (working volume 5L bioreactor). Fermented media were anaerobic with nitrogen sparsing and sterilized by autoclaving at 120°C for 15 min. The ph was maintained at 6.5 with 1 N HCL and 1 N NaOH. The fermentation temperature was kept constant at 30°C.

### IV. Analytical methods

Samples withdrawn at different intervals during growth and fermentation were centrifuged at 10,000 rpm for 10 min. The collected samples were stored at 4 c prior to analysis. Ethanol production and glucose and xylose utilization were analyzed using HPLC (Agilent Technology) using HiPlex – Column at 57°C with 1mM sulfuric acid as the mobile phase at a flow rate of 0.7 ml $min^{-1}$ detected by Refractive Index Detector at 50°C The yeast biomass was determined by its OD value at 600 nm using a spectrophotometer.

### V. Result and Discussion

Yeasts which are the most common micro-organisms in bioethanol production play important role in fermenting sugars to ethanol. Many types of yeasts strains have been identified all over the world with the ability to producing ethanol from different types of feedstocks. .This study aimed to improve the rate of the conversion of the saccharification liquid obtained from algal wastes, containing a mixture of pentose and hexose, to the bioethanol, by selecting pichia stiptis and *Candida Shehatae*, which is generally used in the cofermentation of glucose-xylose. *Pichia Stipitis*, which is used in the fermentation of pentose, such as xylose. Initially, the respective fermentation properties of *Candida Shetatae* and *P. Stipitis*, which have different growth conditions, were investigated, with the co-fermentation properties also examined with respect to the mixing ratio of the xylose and glucose sugars. In the fermentation using *pichia stiptis*, the result of the fermentation under anaerobic conditions was better than under aerobic conditions. In the anaerobic fermentation, the concentration of the reducing sugar and glucose remaining after 96 hrs was 9.09 and 2.88 g/ L, respectively, with 40.59 g/L of ethanol produced; the ethanol productivity was 1.69 g/L-h. In the fermentation using *Pichia Stipitis*, the reducing sugar and glucose rapidly decreased until 120 hrs, with marked ethanol production. However, after 72 hrs, a moderate decrease was observed in the amount of the carbon source, but almost no ethanol was produced. Even after 168 hrs, glucose still remained in the fermentation liquid, with less ethanol produced than in both the anaerobic and aerobic fermentations using *Candida Shehatae.* . As a result, 48.63 g/L of ethanol was produced, with an ethanol productivity of 2.03 g/L-h. This study could be used to help produce a high concentration of ethanol from biomass, wherein monosaccharide and disaccharide, as well as glucose and xylose are contained in the saccharification liquid from algal wastes.

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